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Endotoxin Toxicity in Rats with 6-Sulfanilamidoindazole Arthritis

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Seven oral administrations of 6-sulfanilamidoindazole (6-SAI) to 10- to 12-month-old rats sensitized the animals to endotoxin, with dosages as small as 2.5 μg causing death in 80% of animals. Endotoxin in a dosage of 3,000 μg was not lethal for nonmedicated control animals. 6-SAI-treated 1-month-old rats were not as sensitive to endotoxin as aged animals. The sulfonamide-induced sensitivity to endotoxin could not be passively transferred and could not be explained by blockade of the reticuloendothelial system or impairment of endotoxin detoxification. 6-SAI administration was associated with both depletion of liver glycogen and lowering of blood glucose concentration without changes in blood lactic acid concentration. Disseminated intravascular coagulation is believed to be involved in the pathogenesis of shock and death as evidenced by: (i) concomitant decreases in plasma fibrinogen concentration and elevations in fibrin degradation products after endotoxin challenge; (ii) protection against lethal actions of endotoxin by pretreatment with heparin. Treatment of 6-SAI-medicated rats with glucocorticoids before endotoxin challenge protected the animals against lethal doses of endotoxin and prevented deposition of fibrin thrombi in the glomerular capillaries.

Daily oral administrations of 6-sulfanilamidoindazole (6-SAI) regularly induces acute arthritis and periarticular inflammation in the ankles and hind paws of aged rats (40, 42, 54). 6-SAI also sensitizes rats to endotoxin which causes shock and death (65). The mechanisms responsible for the enhanced toxicity to endotoxin are unknown; however, fibrin deposition has been identified in the viscera and inflamed paws of these animals. The current studies were undertaken with a twofold purpose: (i) to further determine the extent to which 6-SAI enhances endotoxin lethality; (ii) to determine the mechanisms by which 6-SAI sensitizes animals to the lethal actions of endotoxin.

MATERIALS AND METHODS

Animals. Holtzman strain male Sprague-Dawley rats were used in all experiments. Except when specified, animals employed in these experiments weighed 500 to 600 g and were 10 to 12 months old.

6-SAI—route, dosage, and number of administrations. 6-SAI in powder form was suspended in 1% methylcellulose, 150 mg/ml, and administered via gavage tube to ether-anesthetized animals in dosages of 250 mg/kg of body weight daily for 7 consecutive days. Control rats received similar treatments with requisite volumes of 1% methylcellulose.

Endotoxin. Lipopolysaccharide B of *Escherichia coli* O55:B5 (Difco) was suspended in sterile, nonpyrogenic 0.85% NaCl and administered in 1.0-ml volumes.

Blood coagulation studies. Blood was withdrawn from the abdominal aorta for platelet counts (45), concentrations of plasma fibrinogen (23) and serum fibrin degradation products (FDPs; 32), and assay of heparin-precipitable fibrinogen. Heparin-precipitable fibrinogen is an altered form of fibrinogen in heparinized plasma that forms a precipitate in the cold which dissolves upon warming of the plasma (61). It consists of intermediates in the conversion of fibrinogen to fibrin, fibrin degradation products, and possibly other plasma proteins (36, 52, 61) and is detected in the blood of humans by ethanol gelation and protamine sulfate precipitation tests. To assay for heparin-precipitable fibrinogen, 4.0 ml of blood was added to 400 U of heparin (sodium heparin, 1,000 U/ml). The plasma was separated by centrifugation, placed in an ice bath, and observed for the presence of a precipitate 4 h later.

Endotoxin sensitivity in aged and young rats. Results of a previous study on limited numbers of animals demonstrated that seven administrations of 6-SAI sensitized rats to endotoxin, with doses of 6.25 μg causing death in some animals (65). In contrast, endotoxin in dosages of 400 μg was not lethal in control animals. These studies are an extension of the previous

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investigation and were designed to determine the magnitude of endotoxin sensitivity in sulfonamide-treated and control animals. Aged animals were divided into five treatment groups and four control groups, with 10 animals per group. Treatment group rats received a single intravenous (i.v.) injection of endotoxin in dosages of 25, 10, 5, 2.5, or 1.0 μg 24 h after the seventh feeding of 6-SAI. Control animals received a single i.v. injection of endotoxin in dosages of 3,000, 2,000, 1,000, or 500 μg 24 h after the seventh feeding of methylcellulose. All surviving animals were killed 24 h after injection. The 50% lethal dose for endotoxin was calculated by the method of Reed and Muench (46).

To determine whether sensitivity to endotoxin is age dependent, groups of 1-month-old rats, weighing between 100 and 125 g, received a single i.v. injection of endotoxin in dosages of 100, 25, or 10 μg after receiving seven feedings of 6-SAI or methylcellulose.

Effect of sulfanilamide and 6-aminoindazole on sensitivity to endotoxin. These experiments were designed to determine whether sulfanilamide and 6-aminoindazole, the components of 6-SAI, alone or in combination, would sensitize rats to endotoxin. Sulfanilamide and 6-aminoindazole were dissolved in propylene glycol, 125 mg/ml, and were administered separately to groups of aged rats, six animals per group, in dosages of 250 mg/kg of body weight daily for 7 days. To test the combined effect of sulfanilamide and 6-aminoindazole, equal parts of the above solutions were mixed and administered to rats in dosages of 250 mg/kg of body weight for 7 days. Twenty-four hours after the last treatments, animals were challenged with endotoxin in dosages of 100, 50, or 25 μg.

Effect of 6-SAI on the hepatic detoxification of endotoxin. Intravenously injected endotoxin is phagocytized by the reticuloendothelial cells of the liver and spleen (9, 58). It has been shown that the liver detoxifies endotoxin in vivo (49) and in vitro (62). To determine whether seven administrations of 6-SAI interferes with the hepatic detoxification of endotoxin, eight medicated and control rats were killed 24 h after the last 6-SAI or vehicle feeding. One percent liver homogenates from these animals were prepared and assayed for the ability to detoxify endotoxin by the technique of Filkins (17). A 9-ml volume of homogenate from each liver was incubated with 15 μg of endotoxin (in 1.0 ml) at 4 and 37°C for 180 min. Rats weighing 300 to 350 g were sensitized to endotoxin by injecting 5.0 mg of lead acetate in 0.5 ml of distilled water in the femoral vein, followed by 1.0 ml of the incubated homogenate in the contralateral femoral vein. Two lead-sensitized animals were used for each homogenate.

Effect of 6-SAI on carbohydrate metabolism. A major factor in determining survival in shock is the depletion of high-energy intermediate compounds (14, 15). Because glucose is a source of these high-energy intermediates, studies on the effects of 6-SAI on liver glycogen and blood glucose concentration are important. Fifty aged rats were divided into treatment and control groups and were killed 24 h after the last of seven feedings of 6-SAI or methylcellulose for determination of liver glycogen (27), blood glucose (55), and blood lactate (2) concentration.

Effect of 6-SAI on the phagocytic activity of the RES. Because some agents which cause blockade of the reticuloendothelial system (RES) also abolish resistance to endotoxins (3), experiments were designed to determine the effects of 6-SAI on the rate of clearance of colloidal carbon by the technique of Biozzi et al (6). Colloidal carbon, 8.0 mg per 100 g of body weight, was administered via the femoral vein to eight anesthetized (sodium pentabarbital, 3.5 mg per 100 g intramuscularly) sulfonamide-treated and control animals 24 h after the last of seven daily feedings of 6-SAI or methylcellulose. Tail vein blood was withdrawn before and at 2, 6, 10, and 14 min after injection. The blood carbon concentrations were determined, and the phagocytic index, K, was calculated.

Role of DIC in endotoxin lethality in 6-SAI-treated rats. Since endotoxin stimulates blood coagulation (39), and since the viscera and inflamed paws of 6-SAI-treated rats dying after endotoxin challenge contained fibrin thrombi (65), studies on disseminated intravascular coagulation (DIC) were undertaken. These studies on the role of DIC in endotoxin lethality consisted of two types of experiments: (i) the demonstration of DIC; and (ii) the protective effects of heparin.

To demonstrate DIC, 32 rats received 6-SAI daily for seven consecutive days. Twenty-four hours after the last drug administration, eight animals were killed. The remaining animals were divided into two equal groups. Half of the animals received 200 μg of endotoxin i.v., whereas the other half received 2,000 U of heparin i.v. followed by 200 μg of endotoxin i.v. Equal numbers of heparinized and non-heparinized animals were killed at 2 and 4 h after injection of endotoxin. Plasma fibrinogen concentration, FDP, heparin-precipitable fibrinogen, and platelet counts were done on all animals. Control animals were killed 24 h after seven oral administrations of 1% methylcellulose for similar blood coagulation studies.

To demonstrate the protective effect of heparin, 16 animals received 6-SAI for 7 days. Twenty-four hours after the last treatments, half of the animals received 200 μg of endotoxin i.v. whereas the other half received 3,000 U of heparin i.v. followed by 200 μg of endotoxin. The animals were periodically observed, and the times of death were recorded. Surviving animals were killed 24 h after challenge. Kidneys were fixed, sectioned, and stained with phosphotungstic acid hematoxylin.

Passive transfer of endotoxin sensitivity. Donor animals received 6-SAI daily for 7 consecutive days and were exsanguinated 24 h after the last feeding. Blood was collected in sodium citrate (1.0 ml of blood per 0.01 ml of 38% sodium citrate), and the plasma was harvested after centrifugation at 3,000 rpm for 20 min. Seven recipient rats received plasma in dosages of either 1.5 or 4.0 ml per 100 g of body weight intraperitoneally followed by 500 μg of endotoxin i.v. 2 h after injection of plasma.

Effect of glucocorticoids on endotoxin lethality and the generalized Shwartzman reaction. Twenty-four hours after receiving the last of seven daily feedings of 6-SAI, 16 rats were divided into a control group and a treatment group. Control animals received 200 μg of endotoxin i.v. Methylprednisolone (Solu-Medrol, The Upjohn Co., Kalamazoo, Mich.) was dissolved in sterile phosphate-buffered saline, 10

mg/ml, and was administered to the treatment group animals subcutaneously in dosages of 10 mg per kg of body weight. Two hours later, these animals were challenged with 200 μg of endotoxin i.v. All surviving rats were killed 24 h after challenge. The kidneys of all rats were fixed, sectioned, and stained with phosphotungstic acid hematoxylin.

RESULTS

Endotoxin toxicity in aged and young rats. In a previous report on limited numbers of aged animals, endotoxin in dosages of 400 μg was not lethal for control rats whereas doses as small as 6.25 μg were lethal for some animals receiving seven feedings of 6-SAI (65). These experiments are a continuation of the previous study. All aged control animals challenged with endotoxin in dosages of 3,000, 2,000, 1,000, or 500 μg were alive 24 h after challenge (Table 1). In contrast, all aged 6-SAI-medicated rats receiving the 25-, 10-, or 5-μg dosages of endotoxin were dead within 24 h after challenge, whereas 2.5- and 1.0-μg dosages of endotoxin were lethal for 8 of 10 and 1 of 10 animals, respectively. The 50% lethal dose of endotoxin in aged rats receiving 6-SAI was 1.57 μg. All aged animals had well defined arthritis at time of endotoxin challenge, and the dorsa of the inflamed paws of many dead animals were grossly hemorrhagic.

Forty percent of the young rats treated with 6-SAI died after challenge with 100 μg of endotoxin. Smaller doses of endotoxin were nonlethal in 6-SAI-treated young rats. None of the young animals treated with 6-SAI had arthritis.

Effects of sulfanilamide and 6-aminoindazole on sensitivity to endotoxin. All aged animals receiving seven administrations of either sulfanilamide or 6-aminoindazole alone or in combination were alive 24 h after challenge with endotoxin in dosages of either 100, 50, or 25 μg.

Effect of 6-SAI on the hepatic detoxification of endotoxin. Liver homogenate endotoxin mixtures from control and 6-SAI-treated animals were lethal for 2 of 16 and 3 of 16 lead-sensitized recipient animals, respectively, after incubation at 37°C for 180 min. In contrast, after incubation at 4°C for 180 min, endotoxin liver homogenates from both control and sulfonamide-treated animals were lethal for 16 of 16 lead-sensitized recipient animals.

Effect of 6-SAI on carbohydrate metabolism. Seven administrations of 6-SAI resulted in depletion of liver glycogen and a significant reduction in blood glucose concentration without changing blood lactate concentration (Table 2). All 6-SAI-medicated animals lost between 10 and 50 g of body weight, whereas the weights of control animals remained unchanged.

Effect of 6-SAI on the phagocytic activity of the RES. Mean concentration of colloidal carbon in the blood of 6-SAI-treated animals was slightly less than that in control animals (Fig. 1). However, the phagocytic index, K, in sulfonamide-treated and control animals was 0.042 and 0.031, respectively, and was not significantly different as evaluated by Student's *t* test.

Role of DIC in endotoxin lethality in 6-SAI-treated rats. Seven administrations of 6-SAI were associated with increases in mean plasma fibrinogen and FDP concentration and with the presence of heparin-precipitable fibrinogen (Table 3). Mean fibrinogen concentrations in the plasma of rats killed 2 and 4 h after endotoxin challenge were 426 and 933 mg per 100 ml less than those of 6-SAI-treated control animals (Table 3, Fig. 2), whereas mean fibrinogen concentrations in the plasma of heparinized rats killed at similar times after endotoxin challenge were 22 and 147 mg per 100 ml greater

TABLE 1. *6-SAI sensitization of rats to endotoxin*

Treatment	Age	Endotoxin (μg)	No. of deaths/no. of rats[a]
1% Methylcellulose orally, 7 times	10–12 months	3,000	0/10
		2,000	0/10
		1,000	0/10
		500	0/10
	4–5 weeks	100	0/10
		25	0/10
		10	0/10
6-SAI, 250 mg/kg orally, 7 times	10–12 months	25	10/10
		10	10/10
		5	10/10
		2.5	8/10
		1	1/10
	4–5 weeks	100	4/10
		25	0/10
		10	0/10

[a] All surviving animals were killed 24 h after endotoxin challenge.

TABLE 2. *Effect of 6-SAI on liver glycogen, blood glucose, and blood lactate concentration*

Treatment	No. of rats	Liver glycogen concn. (mg % [wet wt])	Blood glucose concn. (mg/100 ml)	Blood lactate concn. (mg/100 ml)
1% methylcellulose orally, 7 times	25	5.28 (1.80–7.81)[a]	82 (61–105)	11 (6–19)
6-SAI, 250 mg/kg orally, 7 times	25	0.457[b] (0.00–2.05)	63[c] (29–118)	15 (7–20)

[a] Numbers in parentheses indicate range.
[b] $P < 0.01$.
[c] $P < 0.05$.

than control concentrations (Fig. 2). Decreases in fibrinogen concentration in non-heparinized endotoxin-challenged animals were associated with increases in concentration of FDPs. However, because blood from heparinized rats would not clot, the FDP concentration could not be determined in the sera of these animals. HPF was present in the plasma of all heparinized rats and in the non-heparinized rats killed 2 h after endotoxin. Platelet counts dropped precipitously after endotoxin challenge and were approximately the same in the blood of both heparinized and non-heparinized animals.

All 6-SAI-medicated rats pretreated with 3,000 U of heparin before endotoxin were alive 24 h after challenge and did not have fibrin thrombi in their glomerular capillaries (Table 4). All non-heparinized animals died 4 to 10 h after endotoxin challenge and had glomerular capillary thrombi.

Passive transfer of endotoxin sensitivity. Endotoxin in dosages of 500 μg was not lethal in rats receiving plasma from 6-SAI-treated donor animals in dosages of either 1.5 or 4.0 ml per 100 g of body weight.

Effect of glucocorticoids on endotoxin lethality and the generalized Shwartzman reaction. All 6-SAI-mediated animals pretreated with methylprednisolone before endotoxin injection were alive 24 h after challenge and were killed. Fibrin thrombi could not be demonstrated in the glomerular capillaries of these animals (Table 5). In contrast, all 6-SAI-treated

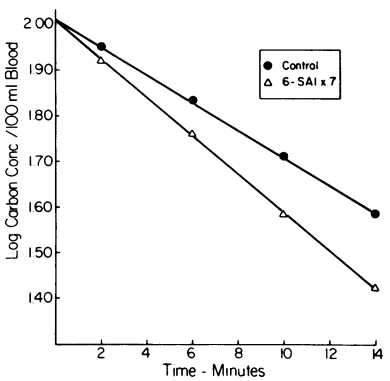

FIG. 1. *Colloidal carbon clearance in 6-SAI-treated and control rats.*

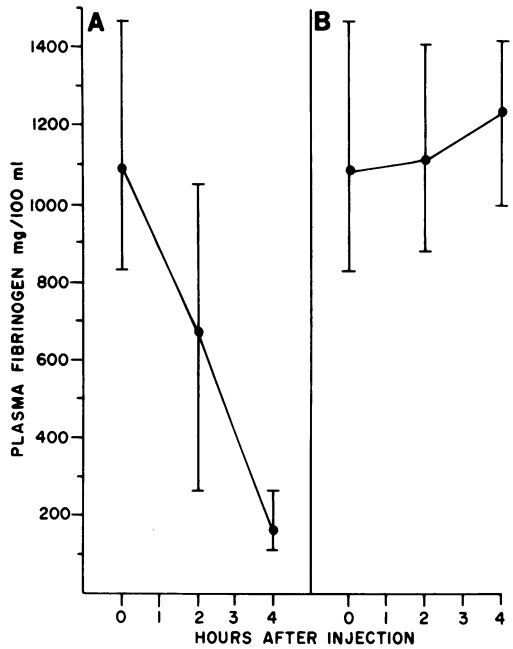

FIG. 2. *Rate of removal of fibrinogen after injection of endotoxin in 6-SAI-treated rats. (A) 200 μg of endotoxin; (B) 3,000 U of heparin and 200 μg of endotoxin.*

TABLE 3. *Effect of SAI and 6-SAI in combination with endotoxin on plasmin fibrinogen concentration, fibrin degradation products, heparin-precipitable fibrinogen, and platelet counts*

Group	Fibrinogen concn. (mg/100 ml)	FDP concn. (μg/ml)	Heparin-precipitable fibrinogen[a]	Platelets ($mm^3 \times 10^3$)
1% Methylcellulose orally, 7 times	352 (326–389)[b]	20 (12–30)	–	1,211 (780–2,200)
6-SAI, 250 mg/kg orally, 7 times	1,094 (834–1,470)	122 (25–200)	+	1,263 (1,132–1,485)
6-SAI, 250 mg/kg orally, 7 times; endotoxin, 200 μg i.v., once; killed at 2 h	668[c] (263–1,050)	230[d] (50–400)	+	259[c] (245–266)
6-SAI, 250 mg/kg orally, 7 times; endotoxin, 200 μg i.v., once; killed at 4 h	161[c] (116–263)	667[c] (400–800)	–	245[c] (220–280)

[a] –, Negative; +, positive.
[b] Numbers in parentheses indicate range.
[c] $P < 0.01$
[d] $P < 0.02$.

TABLE 4. *Effect of heparin pretreatment on endotoxin lethality and the generalized Shwartzman reaction in 6-SAI-treated rats*

Treatment	No. of deaths/no. of rats[a]	No. of rats with glomerular capillary thrombi
6-SAI, 250 mg/kg orally, 7 times; endotoxin, 200 μg i.v., once	8/8	8
6-SAI, 250 mg/kg orally, 7 times; heparin, 3,000 U i.v., once; endotoxin, 200 μg i.v., once	0/8	0

[a] All surviving animals were killed 24 h after endotoxin challenge.

TABLE 5. *Effect of methylprednisolone on endotoxin lethality and the generalized Shwartzman reaction in 6-SAI-treated rats*

Treatment	No. of deaths/no. of rats[a]	No. of rats with glomerular capillary thrombi
6-SAI, 250 mg/kg orally, 7 times; endotoxin, 200 μg i.v., once	8/8	8
6-SAI, 250 mg/kg orally, 7 times; methylprednisolone, 10 mg/kg subcutaneously, once; endotoxin, 200 μg i.v., once	0/8	0

[a] All surviving animals were killed 24 h after endotoxin challenge.

control animals were dead within 24 h after endotoxin challenge and had fibrin thrombi in their glomerular capillaries.

DISCUSSION

The synergistic and lethal interactions between endotoxin and chemicals and drugs has been of interest since the demonstration that lead acetate enhanced the susceptibility of rats to endotoxin by 100,000-fold (50). Cadmium acetate (10), alkylating agents (33), interferon inducers (34, 44), and inhibitors of protein and ribonucleic acid and deoxyribonucleic acid synthesis or metabolism (5, 7, 8, 37, 47, 48) have been reported to enhance the lethal actions of endotoxin. The latter group of drugs includes antibiotics. 6-SAI is the only antibacterial sulfonamide which has been reported to sensitize rats to endotoxin. In addition to inducing arthritis (40, 42, 54) and sensitizing rats to endotoxin (65), 6-SAI also enhances the toxicity of colchicine (M. L. Miller, C. O. Samuelson, and J. R. Ward, Fed. Proc. 37:681, 1978), which may produce a consumptive coagulopathy, and several other drugs (54). Results of recent in vitro and in vivo studies demonstrated that 6-SAI also impairs metabolism of drugs (59). Several other drugs which enhance endotoxin toxicity also interfere with drug metabolism (37, 44).

Results of these studies confirm those of a previous investigation that 6-SAI sensitizes rats to endotoxin (65). Seven administrations of 6-SAI enhance the lethal actions of endotoxin by greater than 1,200-fold. Young animals receiving seven drug treatments did not develop arthritis and were not as sensitive to endotoxin as aged animals. A significant number of young medicated animals challenged with relatively small doses of endotoxin died, thus suggesting that the drug and not the disease is responsible for sensitivity to endotoxin.

Failure of the components of 6-SAI—6-aminoindazole and sulfanilamide—either alone or in combination, to enhance sensitivity to endotoxin demonstrates that the intact 6-SAI molecule or one of its metabolites is responsible for causing increased sensitivity to endotoxin. Results of these studies also demonstrated that sensitivity to endotoxin cannot be passively transferred in the setting employed.

Although a number of inorganic and organic compounds markedly sensitize animals to the lethal actions of endotoxin, there is little agreement as to the mechanism of sensitization. It has been reported that serum factors contribute to the host defenses against endotoxicosis by detoxification of endotoxin (56, 57). The possibility of 6-SAI treatments suppressing endotoxin detoxification by the serum was not evaluated in these studies. Cells of the RES are also sites of sequestration (9, 58) and detoxification (62) of endotoxin. Because blockade of the RES by agents such as Thorotrast induces endotoxin hypersusceptibility (3) and prepares for the generalized Shwartzman reaction (21), experiments were designed to determine whether 6-SAI caused blockade of the RES. Results of these studies demonstrated that seven administrations of 6-SAI were not associated with changes in phagocytic activity of the RES. These observations are of further interest in that there now appears to be little relationship between phagocytic activity of the RES and susceptibility to the lethal actions of endotoxin. Stimulation of the RES by zymosan (4) and glucan (12) are associated with hypersusceptibility to endotoxin, whereas stimulation of the RES with di-

ethylstilbestrol (64) and estradiol (13) is not associated with enhanced susceptibility to endotoxin. Depression of the RES by methyl palmitate decreases endotoxin sensitivity (12). It has been reported that lead acetate enhances (63) and depresses (51) the phagocytic activity of the RES.

Because cells of the RES are involved in both the detoxification of endotoxin (62) and conjugation and acetylation of sulfonamides (24), experiments were designed to determine whether 6-SAI administrations interfered with detoxification of endotoxin by the Kupffer cells. The mechanism seems unlikely since the hepatic extracts of sulfonamide-medicated animals were as effective in inactivating endotoxin as those of control animals.

Alterations in carbohydrate metabolism are associated with host defenses against shock. The early responses to endotoxin are marked by hyperglycemia due to hepatic glycogenolysis, whereas the late stages of endotoxin shock are often associated with hypoglycemia (28). Hypoglycemia is due to an inability to conduct adequate gluconeogenesis after depletion of glycogen stores. The transition of the liver from a glycogenolytic to a gluconeogenic glucoregulatory organ is a critical stage in the metabolic adaptation to endotoxin shock. Treatments which depress gluconeogenesis (16, 20, 53) sensitize to shock, whereas treatments such as glucocorticoids (1, 26, 43) and overnight fasting (19) stimulate gluconeogenesis and increase resistance to endotoxin. Because medicated animals lost some body weight, it is possible that the reduction in liver glycogen and blood glucose concentrations resulted from starvation. It is also possible that 6-SAI is another agent which sensitizes to shock by suppressing gluconeogenesis.

In addition to causing shock, bacterial endotoxins are procoagulants which initiate blood coagulation intravascularly (39). However, the role of DIC in the pathogenesis of shock varies with both the animal species and the experimental model or manipulation. If DIC is an important factor in the pathogenesis of shock, then anticoagulant therapy should significantly reduce mortality. In the classical generalized Shwartzman reaction, which is elicited in young rabbits by two spaced i.v. injections of endotoxin, administration of anticoagulants before the second or provocative injection prevents deposition of fibrin thrombi in the renal glomeruli and protects the animals against shock (22). In contrast to the two-stage Shwartzman reaction, the effects of anticoagulant therapy in other forms of endotoxin shock are controversial. Heparin pretreatment does not protect rabbits against the lethal actions of meningococcal toxin (22). It has been reported (25) and denied (35) that heparin has beneficial effects in canine shock. Pyran enhances endotoxin toxicity in mice; however, heparinization of pyran-treated mice only slightly prolonged survival time after endotoxin challenge (44). Correction of the DIC with heparin in patients with gram-negative bacteremia and DIC does not decrease the mortality rate (11). However, in studies on both nonpregnant and pregnant rats challenged with large doses of endotoxin, it was concuded that, in comparison to other animal species, intravascular coagulation plays an important role in shock in rats because fibrinogen is rapidly removed from the circulation before death and heparin pretreatment prevents a lethal outcome (38). It has also been reported that administration of heparin to normal rats either simultaneously with or at intervals up to 2 h after endotoxin challenge significantly reduced lethality (17). Fibrin thrombi have been demonstrated in the glomerular capillaries of endotoxin-challenged rats pretreated with lead acetate (50), tilerone (34), and 6-SAI (65). Results of these studies on 6-SAI-medicated rats demonstrated that the fibrinogen concentrations in the plasma of 6-SAI-treated animals were approximately threefold greater than those of control animals. These studies also demonstrated that endotoxin induced a massive DIC as evidenced by precipitous drops in blood platelet counts and plasma fibrinogen concentration, and elevations in fibrin degradation products. Because most animals receiving seven feedings of 6-SAI had severe arthritis, the hyperfibrinogenemia may have been a manifestation of inflammation. However previous studies have demonstrated that subcutaneous injections or oral administrations of 6-SAI to aged rats were associated with hyperfibrinogenemia which antedated the onset of clinical disease (41). It cannot be suppressed by hypophysectomy. The hyperfibrinogenemia in 6-SAI-treated rats is of further interest in that it is associated with both increased blood viscosity and failure of red blood cells to sediment in sedimentation tubes (M. L. Miller, C. O. Samuelson, and J. R. Ward, J. Rheumatol., in press). Oral administrations of 6-SAI to 1-month-old rats was not associated with hyperfibrinogenemia or arthritis (41). These studies on endotoxin-challenged arthritic rats also demonstrated that heparin pretreatment prevents the removal of fibrinogen from the circulation, protects against shock and death, and inhibits the generalized Shwartzman reaction.

Results of past and recent investigations have demonstrated that glucocorticoids protect against endotoxin shock (1, 26, 43) and either

prepare for or suppress the generalized Shwartzman reaction, depending upon the duration and timing of steroid administration. Cortisone (60) and the potent synthetic glucocorticoid, triamcinolone (31), prepare for the generalized Shwartzman reaction when given to rabbits in large doses for 3 to 4 days before the provocative injection of endotoxin. However, if a single large dose of hydrocorticone or one of the most potent synthetic glucocorticoids (triamcinolone, dexamethasone, prednisolone) is administered to endotoxin-prepared rabbits or pregnant rats 2 h before the provoking injection of endotoxin, the Shwartzman reaction is decreased in rabbits and completely suppressed in rats (30). In contrast to glucocorticoids, the mineralocorticoid, desoxycorticosterone, is ineffective in either preparing for (31) or suppressing (30) the generalized Shwartzman reaction. Results of the current investigation demonstrated that glucocorticoids protected the sulfonamide-medicated animals against the lethal actions of endotoxin and inhibited deposition of fibrin thrombi in the glomerular capillaries. Results of recent in vivo studies on the mechanisms of the antithrombotic actions of glucocorticoids in endotoxin-challenged rats demonstrated that they prevent DIC through interference with Hageman factor activation and the availability of platelet procoagulant activity (29).

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